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The Verticillium Disease of Chrysanthemum

by

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[1] In cooperation with the State Committee on the Relation of Electricity to Agriculture.
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The Verticillium Disease of Chrysanthemum

By G. A. Huber and L. K. Jones[1]

Introduction

The presence of the Verticillium disease of chrysanthemum in the Pacific Northwest, especially in the Spokane Valley and several sections on the coast, has become quite annoying to the commercial growers. Although some varieties show complete resistance, others show from one to 20 per cent infection, while in a few cases certain susceptible varieties show from 50 to 100 per cent severe defoliation and even complete loss of all the plants in entire beds.

The disease is commonly known as "Verticillium Wilt" or just "Wilt" by the commercial growers. However, in literature the term "Hadromycosis" has come into general usage for all the diseases of plants caused by the vascular Verticillia.

The diseases caused by the vascular Verticillia occur in various countries on many economic, ornamental, and weed hosts. Rudolph (9) gives a table in which he lists 136 plants belonging to 38 families and 18 orders that have been reported in the literature as "susceptible to attack by *Verticillium albo-atrum* and its related forms." Ludbrook (6) made isolations and obtained cultures of Verticillium from 20 different hosts belonging to 17 genera which were obtained from "13 states of the United States, 4 European countries, and 4 states of Australia." Wollenweber (12) reports over 70 plant genera that are known to be attacked by Verticillium species. Van der Meer (10) lists 81 species of dicotyledons that are attacked by the Verticillia.

The history and literature pertaining to Verticillium hadromycosis are treated quite thoroughly in recent publications (6, 9, 10) and need not be repeated since the work here has been confined to the disease of chrysanthemum.

Mention of the Verticillium disease of chrysanthemum has been made by various workers, but no investigation known to the writers has been reported other than the isolation of the organism and the proof of its pathogenicity. The disease was reported (4) as occurring in New Jersey

[1] The writers express their appreciation to Dr. F. D. Heald for his helpful criticism during the progress of the investigations and the preparation of the manuscript.

in 1923 and attributed to *Verticillium albo-atrum* R. and B. Martin (7) again reported the disease in 1925 in the same state. He states that the trouble had been "under observation during the past 6 years, both on plants grown under glass and outdoors." The disease was also reported (8) to be of considerable importance in Michigan in 1926, severe losses occurring in some of the greenhouses. Van der Meer (10) includes *Chrysanthemum indicum* Cass. and *C. leucanthemum* L., in his list of ornamental plants and weeds that showed symptoms of "Verticilliosis." He refers to the organism which was isolated from these plants as *Verticillium dahliae* Kleb. In 1929, Wollenweber (11) included *Chrysanthemum indicum* Cass. in his list of hosts attacked and placed the organism causing the disease in the *Verticillium ablo-atrum* group. In 1932, the writers (5) reported the disease as being prevalent in greenhouses in the state of Washington, and considered the organism isolated from the diseased plants to be *Verticillium dahliae* Kleb.

Symptoms

The term "wilt" does not always apply to the Verticillium disease of chrysanthemum, since a non-wilting type of symptom prevails in abundance.

When a plant responds to the infection by a general wilting, the leaves become paler in color, then turn brown and die, and remain attached to the stem. In severe cases all the foliage wilts and the blossom bud fails to open. (Plate I).

When the plant does not respond to the infection by a general wilting (Plate II), the sequence of development of the symptoms is as follows: The lower leaves show the first response, interveinal areas becoming pale green or yellowish green and finally brown (Plate III), followed by the extension of the necrotic areas, the green portions remaining normal, until the entire leaf is finally involved. Brown necrotic areas appear on the petiole soon after the symptoms first show on the leaf. These symptoms develop progressively upward from the lowest leaves as the organism advances up the stem, while the dead leaves droop and remain attached. All degrees of infection may be exhibited from plants showing only a few of the lower leaves involved to the extreme condition in which all the leaves are killed and the blossoms turn brown and die.

Infected plants become stunted and the flowers are reduced in size, depending on the severity of the attack, and the quality is greatly lowered. In the Golden Measure variety the average diameter of 12 flowers from healthy plants was 6.4 inches, while the average for 12 flowers of diseased plants, symptoms of the non-wilting type showing up half their height, was 4.3 inches.

In severe cases the organism advances up the stem into the base of the flower, and the petals turn brown and die (Plate II, B).

External black to brown streaks extending up the stem have been observed only in a few cases. However, when the stems of diseased plants are cut just beneath the point of attachment of leaves showing large necrotic areas, a slight discoloration of the woody tissue is evident.

The roots show no symptoms with the exception of drying up as the plant dies.

Cultural Studies

Isolation

During the progress of the investigation, isolations were made from over 100 chrysanthemum plants of different varieties coming from various parts of the state.

In the earlier part of the investigation difficulty was encountered in obtaining the organism because of the presence of a rapidly spreading bacterium which usually accompanied the isolations. The bacterium was quite resistant to the usual method of acidifying the medium for retarding bacterial growth, and overgrew the slowly-developing Verticillium mycelium. It also resisted the usual method of dipping infected host parts into 70 per cent alcohol followed by passing through a flame.

The method which proved satisfactory for isolating the causal organism and eliminating bacterial development was as follows: The infected host part was placed in 70 per cent alcohol from eight to 10 seconds and then transferred to a solution of mercuric bichloride (1-1000) for 30 seconds to one minute. The host part was then dried with a piece of absorbent cotton which had previously been saturated with mercuric bichloride (1-1000) and squeezed partially dry. The diseased host part was then cut open with a sterile knife and portions of the inner woody tissue transferred to potato dextrose agar plates. In no case was there a sufficient amount of the mercuric bichloride carried with the tissue transfers to prevent the development of the fungus.

Causal Organisms

The taxonomic treatment of the two vascular Verticillia, *V. albo-atrum* R. and B. and *V. dahliae* Kleb., which has been discussed by many investigators, is based primarily on the absence or presence of "microsclerotia" in culture. Wollenweber (11) and Rudolph (9) consider that the presence or absence of "microsclerotia" is an unreliable character and that the two forms should be classified as *Verticillium albo-atrum*. On the other hand, Van der Meer (10), Berkeley, Madden and Willison (2), and Ludbrook (6) support the views of Klebahn and classify the 'microsclerotial" producing forms as *Verticillium dahliae* Kleb. Berkeley et al. (2) made a cultural and taxomonic study of many strains of Verticillia associated with hadromycosis and divided them into two groups: (1)

V. albo-atrum group which produces the "resting condition in the form of darkened, many septate, torulose hyphae, but lose the power of producing this resting stage under continued culturing on artificial media"; and (2) *V. dahliae* group which produces the resting condition "in the form of mono-hyphal pseudo-sclerotia and most members of the group do not lose the power of producing pseudo-sclerotia under conditions of continued culturing on arificial media."

Comparison of cultures of these two forms, and the study of the cultures isolated from diseased chrysanthemums, as well as a study of the literature of various investigators, lead the authors to conclude that the causal organisms of the "Verticillium disease of chrysanthemum" under investigation belong to the *V. dahliae* group. One of the cultures which was isolated from a diseased chrysanthemum plant in 1929 was sent to Ludbrook and was identified by him (6) as *V. dahliae.*

Two apparently distinct types of *V. dahliae* according to cultural characters have been isolated from chrysanthemum plants. No difficulty has been encountered in making a sharp distinction between the two types in any of the cultures obtained. Several isolations from diseased plants showed that both types of cultures may be obtained from the same plant; however, they could be separated by single pseudo-scrlerotial isolations.

All the cultures were carried in stock on potato dextrose agar slants in the low temperature chest and transfers were made every two months. A representative of each type (Cultures No. 14 and No. 26) was selected for cultural and inoculation study.[1] They were grown in parallel culture on various culture media.

Growth on potato dextrose agar. The cultures show the greatest differences on this medium. Culture No. 14 produces numerous individual pseudo-sclerotia in rings or zones (Plate IV, A), but these are absent when cultures are grown in total darkness, other conditions being similar. The pseudo-sclerotia begin development by the formation of one to several short thick-walled cells within the mycelium. These increase in number by budding, forming dark thick-walled cells which become heaped into separate compact masses (Plate V, A, B, C). The mycelium is mostly medium-living with a very slight aerial growth of vegetative hyphae at the center of the colony. Sporulating hyphae are abundant over the entire colony.

With culture No. 26, the medium-living mycelium becomes changed into a series of more or less spherical, linked cells through transverse septa (Plate V, D). These cells soon multiply by budding to produce a large number of lateral cells (Plate V, E, F, G) and so increase to form a compact mass, or crust, over the surface of the medium (Plate IV, D)

[1] No attempt is made in the present paper to go further than to point out a few cultural and pathogenic differences in the two types.

which generally becomes wrinkled or buckled. The crust is generally covered with very loose aerial soprulating hyphae, with a patch of dense, white, smooth, cottony mycelium at the center of the colony, which sometimes extends toward the margin. The crust also forms when cultures are grown in total darkness.

Both types of cultures showed a tendency to produce a more copious growth of aerial mycelium with age, but by successive transfers every seven or eight days, the original characters were restored.

Growth on prune agar. Culture No. 14 (Plate IV, B) produces an abundance of pseudo-sclerotia, almost forming a crust, while culture No. 26 (Plate IV, E) produces a compact mass or crust which becomes more or less heaped above the surface of the medium.

Growth on corn meal agar. Culture No. 14 (Plate IV, C) produces very little or no aerial vegetative mycelium. The pseudo-sclerotia are produced around the initial inoculum in the center of the colony, but rings or zones of pseudo-sclerotia are not produced. Culture No. 26 (Plate IV, F) produces a dark crust very similar to the one formed on potato dextrose agar; however, the crust is not so compact or wrinkled.

Inoculation Studies

The same representative cultures as used in the cultural studies were used in the inoculation tests. The inoculations were made into the stems of well-rooted cuttings growing in six-inch pots. The soil was removed from the base of the stems and the inoculum (spores and mycelium from plate cultures) was introduced into wounds made just beneath the ground level by a sterile scalpel. After inoculation, the soil was replaced to prevent drying. When the organisms were well established, the plants were transplanted to ground beds, the soil of which was new to chrysanthemums.

Table 1. Results of inoulation tests showing the varieties attacked by the two types of *V. dahliae*

Culture No. 14 only	Culture No. 26 only	Both cultures (No. 14 and No. 26)
Friendly Rival	Monument	Chadwick (very severe by No. 26)
Turner	Oconto (100%)	Chieftain
	Pink Mistletoe	Dr. Enguehardt
		Golden Measure
		Seidewitz (very severe by No. 26)
		Whittier

Six to eight plants of each variety were inoculated with each of the two types of oragnisms. The percentage of plants diseased, including those atacked by both types, is given in Table 3. Table 1 shows the varieties attacked by each type and those attacked by both types.

Although the varieties Chadwick and Seidewitz show 100 per cent susceptibility when inoculated with either type of culture, the disease is much more severe when the plants are attacked by No. 26. In the greenhouse observations where these two varieties were severely affected by the disease, either both types or only No. 26 was obtained when isolations were made. Those varieties that showed susceptibility to only one type in the inoculation tests always revealed the presence of the same type in isolations from naturally infected plants.

The Relation of Pathogene to Host Tissue

Workers on various Verticillium problems have generally agreed that infection takes place through the roots, but they have differed in opinion concerning the exact methods by which the organisms can enter the host.[1] Some have found that both injured and uninjured roots could be attacked readily, while others were unable to secure infection through the uninjured roots.

In the present investigation inoculations through wounds gave, in susceptible varieties, 100 per cent infection and rooted cuttings transplanted to inoculated soil gave approximately the same amount of infection; however, not nearly so severe. From these results, one can definitely state that the organisms gain entrance through wounds, but there are no data available to ascertain that infection takes place through uninjured roots, although it seems highly probable.

According to Van der Meer (10), after the organism gains entrance into the roots, "Verticillium grows from the wood vessels of the roots or other subterranean parts through the tracheae and tracheids of stem or trunk in upward direction, penetrates the wood vessels of lateral stems or branches, and spreads through the vessels of the petiole into the network of veins."

Longitudinal sections of chrysanthemum stems from both naturally infected and wound-inoculated plants revealed the presence of hyphae in the wood vessels, and most generally in those closest to the pith. Sections made from the stems of inoculated plants showing severe symptoms (these were made at three-inch intervals from the point of inoculation to the growing tip of the plant) showed the presence of the fungus. Isolations made at two-inch intervals from similar plants gave the organism in every case. Isolations made from the base of diseased flowers showing partial browning, and from petioles and main veins of leaves showing necrosis

[1] Van der Meer (10) and Rudolph (9) give a review of this phase of the subject.

EXPLANATION OF PLATES

PLATE I. Chrysanthemum plants: one healthy (left) and three diseased plants showing the wilting type of symptom.

PLATE II. Chrysanthemum plants: A, healthy; B, entire plant showing the non-wilting type of symptom; C, lower portion of a plant showing the non-wilting type of symptom.

PLATE III. Chrysanthemum leaves: one healthy (upper left) and four leaves from infected plants showing necrotic areas.

PLATE IV. Plate cultures of the two forms of *V. dahliae* isolated from chrysanthemum plants: Culture No. 14 on A, potato dextrose agar, B, prune agar, C, corn meal agar. Culture No. 26 on D, potato dextrose, E, prune agar, F, corn meal agar.

PLATE V. Photomicrographs of the two forms of *V. dahliae* isolated from chrysanthemum plants: Fig. A-C, Culture No. 14. A, early stage of pseudo-sclerotium formation, x250; B, pseudo-sclerotia in outer zone of colony, x50; C, pseudo-sclerotia in inner zone of colony. Fig. D-G, Culture No. 26. D, early stage of pseudo-sclerotium formation, x250; E, showing beginning of lateral budding, x250; F, later stage showing budding, x250; G, near edge of colony showing pseudo-sclerotia.

PLATE I

PLATE II

Plate III

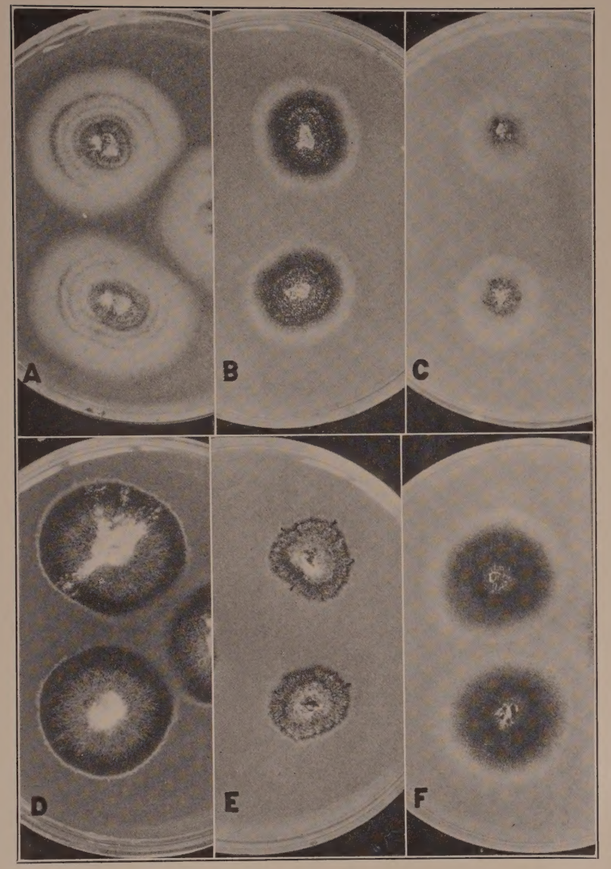

Plate IV

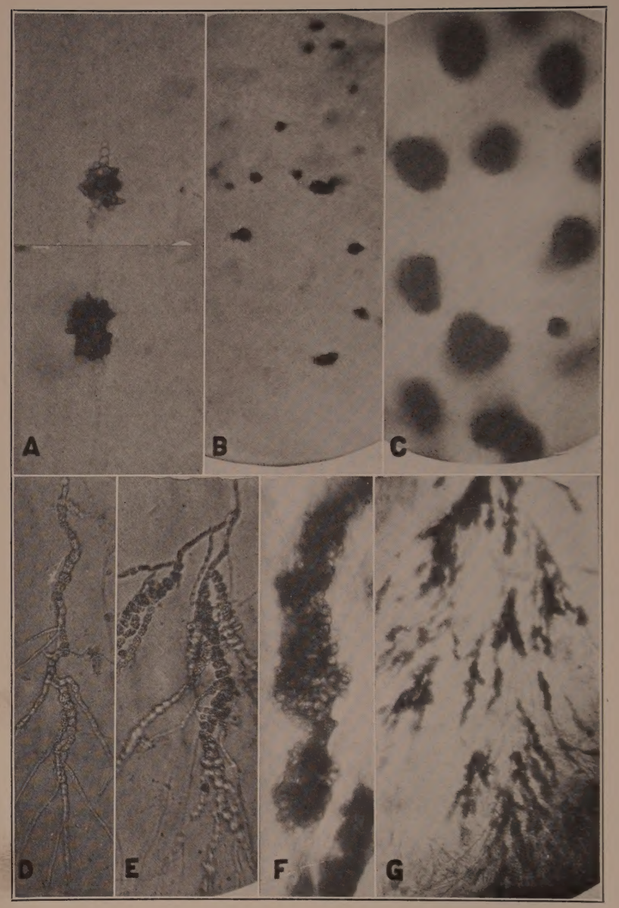

Plate V

gave the causal organism. Neither sections nor isolations revealed the organism two or more inches below the point of inoculation. Longitudinal sections made from infected stems one and one-half to two inches above the last leaf showing symptoms revealed the presence of hyphae. That the organism is in advance of the symptoms on the leaves was further verified by obtaining the organism through isolations two inches above the highest leaf showing symptoms on the stem. This fact was also demonstrated by obtaining the organism from cuttings showing no symptoms.

Spread of the Disease

Observations during several years indicated that cuttings from stock plants which carry the organism are the main factors responsible for carrying the disease from one crop to the next, and for the spread of the disease from one greenhouse to another. For example, one year the Verticillium disease was absent in a greenhouse growing several susceptible varieties. The following year, some of the varieties showed as high as 50 per cent diseased plants. Upon questioning the grower, it was found that the cuttings were obtained from another grower in whose greenhouse the disease was very abundant. A more convincing example is a case where the grower steam sterilized the soil each time before transplanting the cuttings which had been previously rooted in sterilized sand. One year the disease was absent and the following year it was very prevalent. The stock plants in the former case showed no disease and were obtained from beds in which the disease was absent, while in the latter case the stock plants were obtained from beds showing an abundance of diseased plants.

In order to test the probability that the cuttings carry the organism, isolations were made from shoots taken from stock plants that showed the presence of the disease in the previous crop. These stock plants, including three susceptible varieties, namely, Chadwick, Golden Measure, and Seidewitz, were planted in the experimental greenhouse, some of each variety being held at 60° F. and the remainder at 70° F. When the shoots were three to five inches in length, they were removed from the parent plants and isolation made from the stem of each shoot at various heights. The results showed very clearly that cuttings taken from diseased stock plants are capable of carrying the organism (Table 2). In some cases the stock plants growing at 70° F. died before shoots of sufficient size for cutting were produced. Ludbrook (6) states that potted plants inoculated with *V. dahliae* became diseased with approximately equal severity at air temperatures from 17° to 28° C. (62.8° to 82.4° F.). In the present experiment the disease appeared to develop more rapidly in the 70° temperature than in the 60° temperature.

From the literature on the occurrence of the Verticillia in the soil, Rudolph (9) states, "Many writers agree that a high content of manure, compost, humus or other organic matter in the soil is conducive to the

Table 2. Isolations from cuttings. Results of isolations made from cuttings taken from stock plants grown in greenhouse compartments held at 60° and 70° F.

Variety	Greenhouse temperature (degrees Farenheit)	No. of cuttings from which isolaions were made	No. of cuttings from which the organism was obtained	Condition of stock plants when planted[1]
Chadwick	60	9	2	Diseased
Chadwick	70	5	4	Diseased
Golden Measure	60	10	0	Healthy
Golden Measure	60	8	1	Diseased
Golden Measure	70	n[2]	0	Healthy
Golden Measure	70	—[3]	—	Diseased
Seidewitz	60	10	3	Diseased
Seidewitz	70	7	7	Diseased

[1] ''Diseased'' indicates that the stock plants showed the presence of the disease the previous year. ''Healthy'' indicates that the stock plants were selected from beds that showed no disease the previous year.
[2] A large number of disease-free cuttings were obtained for various tests.
[3] The stock plants were killed before shoots of sufficient size for cuttings were produced.

spread and propagation of the fungus." Apparently the fungus will persist in soils varying from a light sandy loam to a heavy clay. Various types of soil sterilization have been tried with varying results. Steam sterilization has been considered as giving the best results in the greenhouse since the fungus is very easily killed by heat. However, Bewley (3) states that the fungus spreads more rapidly in sterilized soil than in unsterilized soil. This emphasizes the importance of using healthy cuttings in propagation.

Varietal Susceptibility

From greenhouse observations and inoculation studies there appear to be marked differences in the resistance of the different varieties to the disease (Table 3). With a few varieties where a large number of beds in various greenhouses have been observed, it is safe to state the relative degree of resistance. However, with other varieties where few beds have been under observation, and no inoculation tests have been made, the degree of resistance is uncertain. Some varieties, as Chieftain, Monument, Oconto, and Turner, showed a greater degree of susceptibility in the inoculation tests than in the observations under commercial growing conditions. The variety Justrite, showed a high degree of susceptibility in one greenhouse two different years. However, no infection of plants of the Justrite variety occurred in the inoculation tests[1] or in other greenhouses in which the disease was present on other susceptible varieties. No explanation can be given since the plants grown in one greenhouse where the disease was prevalent were not tested by inoculation. However, culture No. 26 of *V. dahliae* was consistently isolated from the diseased Justrite plants.

From observations in greenhouses and on the basis of inoculation tests it is concluded that the following varieties are resistant to attack by this organism: Adrian's Pride, Ambassador, Antique, Betsy Ross, December Beauty, December Glory, Early Rose, Early Frost, Gold Lode, Golden Bronz, Golden Glory, Golden Queen, Honey Dew, Indianola, Italian King, Lustre, Marie D. Peters, Maude Dean, Missoula White, Mrs. Alex Laurie, Mrs. Calkins, Monument, Pink Treasure, and W. H. Waite. The following commonly grown varieties are extremely susceptible to injury: Chadwick, Distinction, Dr. Enguehardt, Favorite, Golden Measure, Justrite, Mrs. Simpson, Seidewitz, and Whittier.

Some of the other varieties observed in greenhouses appear to be resistant to the disease. However, sufficient observations upon which to base conclusions have not been made on these apparently resistant varieties.

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[1] Ten rooted cuttings were inoculated with each of the two types of Verticillium and all the plants remained healthy. The stock plants were obtained from a greenhouse showing no disease in that variety.

Table 3. Varietal Resistance. Results based on greenhouse observations and inoculation tests

Variety	Greenhouse observations		Inoculation tests	Variety	Greenhouse observations		Inoculation tests
	No.	Per cent diseased	Per cent diseased		No.	Per cent diseased	Per cent diseased
Adrian's Pride	2	0	0	Justrite	7	0-100	0
Ambassador	2	0		Lustre	6	0	0
Antique	2	0		Margaret Graham	1	0	
Betsy Ross	2	0		Marie D. Peters	3	0	
Bonaffan	5	0-5		Maude Dean	2	0	
Chadwick	15	0-50	100	Missoula White	1	0	
Chieftain	9	0-1	40	Mrs. Alex Laurie	2	0	
Clara B. Ford	1	0		Mrs. Calkins	2	0	
December Beauty	6	0	0	Mrs. Robinson	1	0	
December Glory	6	0-1	0	Mrs. Simpson	4	2-100	
Dorothy Turner	1	100		Monument	8	0	6
Early Rose	1	0		Nellie T. Ross	1	0	
Early Frost	1	0	0	Oconto	3	0	40
Dr. Enguehardt	7	0-100	100	October Frost	1	-1	0
Fantan	2	0-2		October Rose	2	0-50	
Favorite	5	0-60		Pink Mistletoe	3	0-20	8
Friendly Rival	11	0-1	5	Pink Reliance	1	0	
Gladys Pearson	3	0-20		Pink Treasure	2	0	
Gold Lode	2	0	0	Pride of Richmond	1	0	
Golden Bronz	1	0		Quaker Maid	1	2	
Golden Glory	2	0		Razor	1	0	
Golden Measure	3	100	100	Rose Chochard	1	0	
Golden Queen	12	0	0	Rose Glory	1	100	
Golden Wave	1	0		Rose Royal	1	25	
Gov. Green	1	0		Seidewitz	16	0-25	100
Helen Frick	1	1		Silver Sheen	7	0-1	
Honey Dew	2	0		Turner	10	0-1	20
Imp. Golden Glow	3	0		Whittier	5	0-25	100
Indianola	10	0	0	W. H. Waite	10	0	0
Italian King	1	0		Wolf's Pink	1	60	

Control Recommendations

Since the mycelium of the Verticillium may be carried in cuttings taken from diseased chrysanthemum stock plants it is well to select the stock during the flowering season. Plants showing no symptoms of the disease should be marked and saved for propagation purposes. It appears that plants from cuttings affected with the disease are, in general, more severely damaged than are plants that contract the disease from the soil following "benching."

The following facts concerning the pathogene, *Verticillium dahliae* Kleb. serve to emphasize the value of and the necessity for steam sterilization of the soil used in chrysanthemum culture: (a) The virgin soil from the forests (especially western Washington) is often contaminated with this organism; (b) the organism attacks a large number of species and varieties of plants and hence may be present in soil which has not previously been used for growing chrysanthemums; and (c) the parasite may persist in the soil for an unknown period of time. Since the fungus is reported to spread more rapidly in sterilized than unsterilized soil, the prevention of infection after sterilization is emphasized.

A large number of varieties have been found to be resistant while others are very susceptible to attacks by this organism. Accordingly, the resistant varieties should be used wherever practical.

Summary

The Verticillium disease ("Hadromycosis" or "Wilt") has caused considerable loss to chrysanthemum growers.

The disease is manifested by a conspicuous yellowing and browning of the leaves followed by their death from the base of the plant upward. Infected plants become stunted and the flowers are reduced in size. In certain cases the affected plants may wilt and fail to develop flowers.

This disease is caused by *Verticillium dahliae* Kleb., a soil-inhabiting fungus, two forms of which were isolated from diseased chrysanthemum plants and their pathogenicity proved by inoculation.

The pathogene gains entrance to the plant through the roots and advances upward in the water conducting tissue.

The organism is often carried in cuttings used for propagation that are made from diseased stock plants.

From observations in greenhouses and from inoculation tests it is concluded that a number of varieties of chrysanthemums are resistant to attack by this organism.

The use of cuttings from disease-free stock plants, steam sterilization of the soil, and the use of resistant varieties are recommended in order to reduce losses from this disease.